Reference: *Biol. Bull.* **176:** 79–95. (April, 1989)

Ultrastructure of the Ovary and Oogenesis in the Ovoviviparous Ophiuroid *Ophiolepis paucispina* (Echinodermata)

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Abstract. *Ophiolepis paucispina* (Say) produces large yolky oocytes and has ovoviviparous reproduction. The ultrastructure of the somatic tissue layers of the ovarian wall and the germinal epithelium of *O. paucispina* is described. Oogenesis is continuous with oocytes at different stages of development interspersed in the ovary. Proliferation of oogonia and initial growth of the primary oocytes occur within a follicle. Thereafter, the oocytes are largely independent of the germinal layer, and oogenesis is solitary. The oocytes bulge outwards as they grow, forming a sac-like depression in the ovarian connective tissue, and are connected to the germinal layer by a short stalk. Subsequently, haemal fluid accumulates in the connective tissue adjacent to each vitellogenic oocyte. Although each oocyte is enveloped by haemal fluid, it is separated from the fluid by the inner basal lamina. Vitellogenesis involves an accumulation of yolk bodies that appear to be formed jointly by the endoplasmic reticulum and the Golgi complex. Endocytotic activity is prevalent throughout vitellogenesis, indicating incorporation of exogenous material by the oocytes. It is suggested that the genital haemal sinus serves as an intragonadal nutrient store and that yolk precursors, potentially a component of haemal fluid, may be sequestered by the oocytes. *O. paucispina* appears to have heterosynthetic yolk formation and the evidence for this mechanism of vitellogenesis in the Echinodermata is discussed.

Introduction

Although the ultrastructure of echinoderm oocytes is described in several investigations, the cellular events of oogenesis and the dynamics of yolk deposition are not well documented (reviews: Piatigorsky, 1975; Walker, 1982; Kanatani and Nagahama, 1983). Most studies of echinoderm oogenesis describe aspects of asteroid and echinoid oocyte development (Piatigorsky, 1975; Walker, 1982; Kanatani and Nagahama, 1983; Beijnink *et al.*, 1984c; Ferrand, 1984; Aisenshtadt and Vassetzky, 1986) and there is a recent investigation of holothuroid oogenesis (Smiley, 1988). For ophiuroids, there are two ultrastructural studies of oogenesis (Kessel, 1968; Byrne, 1988), and several thesis reports on the ultrastructure of the ovary and the oocytes (Patent, 1968; Davis, 1971; Bell, 1974; Tyler, 1976).

This study describes the ultrastructure of the ovary and oogenesis of *Ophiolepis paucispina* (Say), a small hermaphroditic ophiuroid (5–7 mm disc diameter) that broods its young in the genital bursae (Hendler, 1979). Like most brooding ophiuroids (Hendler, 1975, 1979; Strathmann and Strathmann, 1982), *O. paucispina* is ovoviviparous and produces a small number (2–20) of large oocytes (400 μm diameter). Embryonic development appears to be completely supported by the nutrient reserves present in the oocyte. In contrast to the viviparous ophiuroid *Amphipholis squamata* (Delle Chiaje), extra-embryonic nutrient reserves are not present in the bursal wall of *O. paucispina* (Fell, 1946; pers. obs.).

Recent interest in echinoderm oogenesis has focussed on oocyte nutrition (Walker, 1982; Voogt *et al.*, 1985). For many echinoderms, it appears that somatically derived nutrients are delivered to the ovary by coelomic transport and stored for oogenesis by the haemal system (Walker, 1982; Ferguson, 1982, 1984; Voogt *et al.*, 1985; Beijnink and Voogt, 1986; Byrne, 1988). The somatic synthesis of vitellogenin has been demonstrated biochemically for echinoids (Harrington and Ozaki, 1986;

Received 22 November 1988; accepted 24 January 1989.

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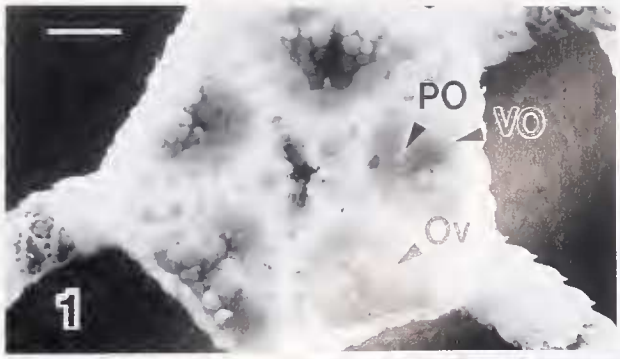
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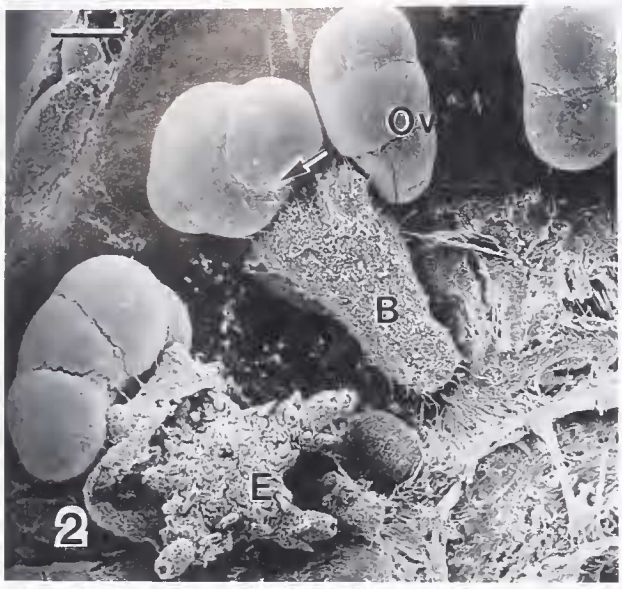
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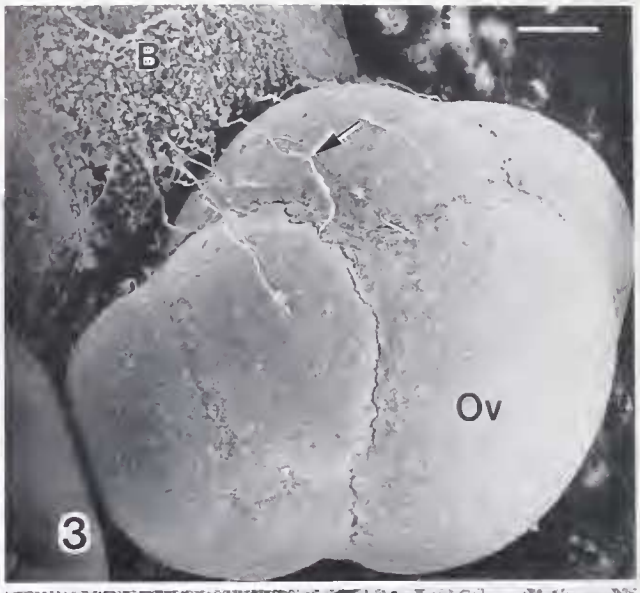
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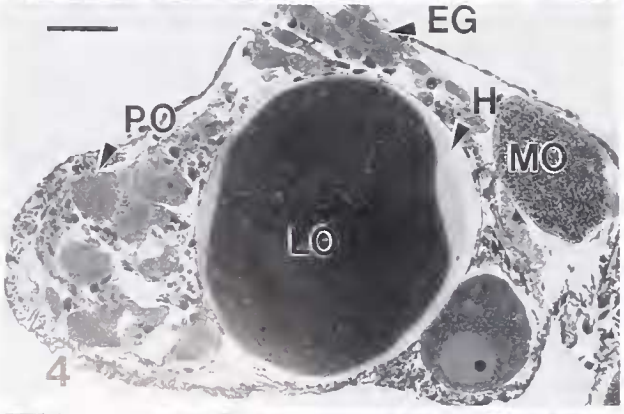
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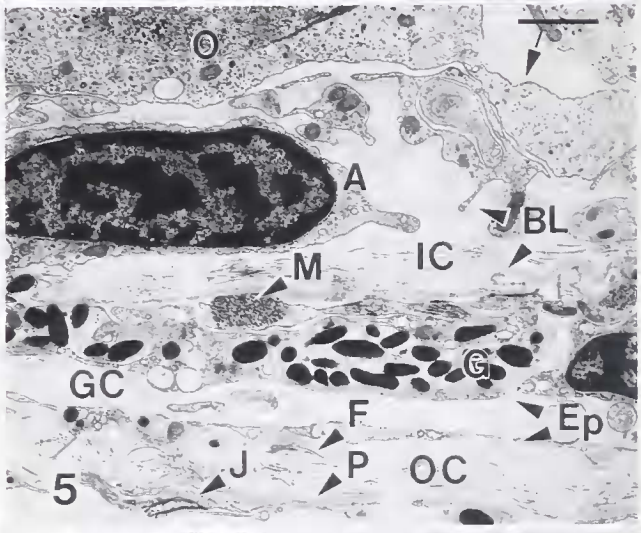
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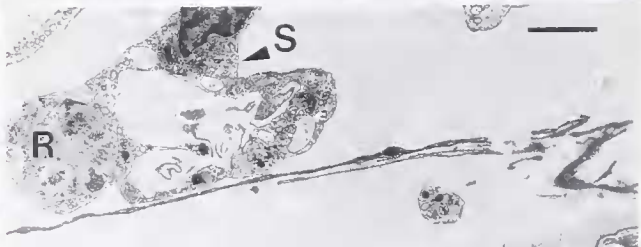
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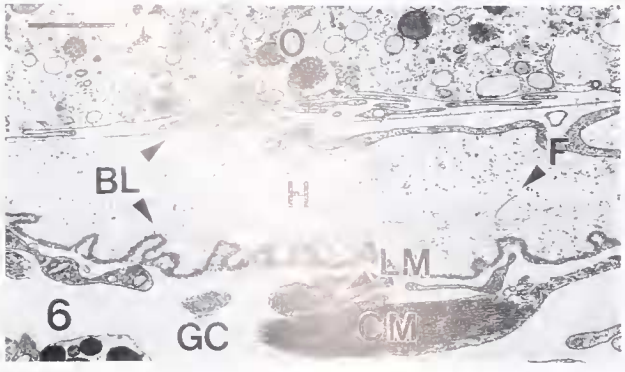
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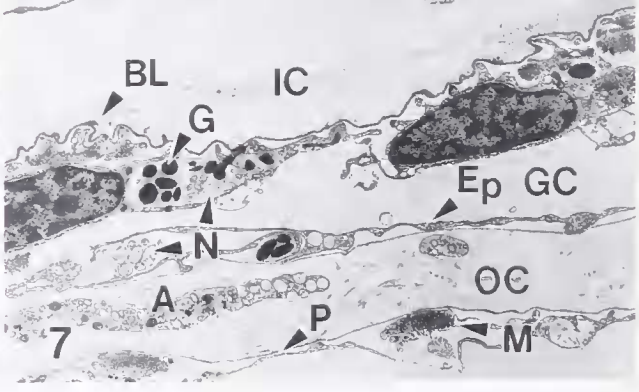
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Shyu *et al.*, 1986). As in other echinoderms (Walker, 1982), the genital haemal sinus of *O. paucispina* contains PAS + material and vitellogenesis involves the elaboration of PAS + yolk, suggesting the incorporation of haemal material by the oocytes (Byrne, 1988). Moreover, the presence of endocytosis by the oocytes indicates uptake of exogenous material (Byrne, 1988). In this investigation, the developmental sequence of oocyte growth in *O. paucispina* is documented from the early proliferative stage through the previtellogenic and vitellogenic stages. Emphasis is placed on the interaction between the germinal and the somatic cells of the germinal epithelium, the role of the genital haemal sinus, and on the vitellogenic mechanisms involved in the production of the yolky oocyte. This is the first such investigation for an ophiuroid and its significance is assessed with respect to current concepts of ovarian structure and oocyte development in echinoderms.

Materials and Methods

Specimens of *Ophiolepis paucispina* were collected at 0.5–1.0 m depth from clumps of the calcareous alga *Halimeda* from Conch Key, Florida (monthly, January through December, 1985), and from Southwater and Carrie Bow Cays on the Belize Barrier Reef (June, 1985, and April, 1986). Specimens for transmission electron microscopy were placed in primary fixative on a cold plate without primary anaesthesia. The ovaries were excised and placed in fresh fixative. Four fixation methods were employed, including phosphate buffered glutaraldehyde (Cloney and Florey, 1968), cacodylate buffered glutaraldehyde (Walker, 1979), and seawater buffered glutaraldehyde (Buckland-Nicks *et al.*, 1984). Osmication followed primary fixation using the same buffer (Cloney and Florey, 1968; Walker, 1979) or sodium bicarbonate (Eckelbarger, 1979; Buckland-Nicks *et al.*, 1984). Best fixation was obtained with the method of Buckland-Nicks *et al.* (1984) as follows: the ovaries were fixed in 2.5% glutaraldehyde in 0.45 μm millipore-filtered natural seawater for 1 h at room temperature. This was followed by a rinse in 2.5% sodium bicarbonate (pH 7.2) and secondary fixation in 2% osmium tetroxide in 1.25% sodium bicarbonate for 1 h at room temperature. Following fixation, the tissue was rinsed in distilled water, dehydrated in ethanol, transferred to propylene oxide, and embedded in Epon 812. The cacodylate method (Walker, 1979) yielded the best preservation of the cortical granules and the vitelline envelope. For light microscopy, 1-μm sections were stained with a mixture of methylene blue and Azur II. Thin sections were stained with 2% uranyl acetate followed by 2% lead citrate and viewed with a Zeiss EM9-S2 electron microscope.

Specimens for scanning electron microscopy were also fixed according to Buckland-Nicks *et al.* (1984), dehydrated in ethanol, transferred to acetone, and critical point dried. The dried specimens were mounted, sputter coated, and viewed with a Novascan 30 or Hitachi S-570 SEM.

Results

Structure of the ovary

The ovaries of *Ophiolepis paucispina* are attached to the ambulacral or interambulacral genital ossicles positioned on either side of the bursal slits and project into the perivisceral coelom (Figs. 1, 2). There are up to three ovaries per bursa ranging in length from 600–1000 μm. Suspensor cells connect the peritoneal lining of the bursa and the ovary wall (Figs. 2, 3). Reproduction is continuous with the ovaries containing oocytes at different stages of development throughout the year (Figs. 1, 4). Vitellogenic oocytes are yolky and pale pink in life, and at the

Figure 1. *Ophiolepis paucispina* with the aboral surface removed to show the ovaries (Ov) containing previtellogenic (PO) and vitellogenic (VO) oocytes. Scale = 1.0 mm.

Figure 2. Scanning electron micrograph (SEM) showing the ovaries (Ov), peritoneal suspensors (arrow), bursal wall (B), and an embryo (E) emerging from a torn bursa. Scale = 300 μm.

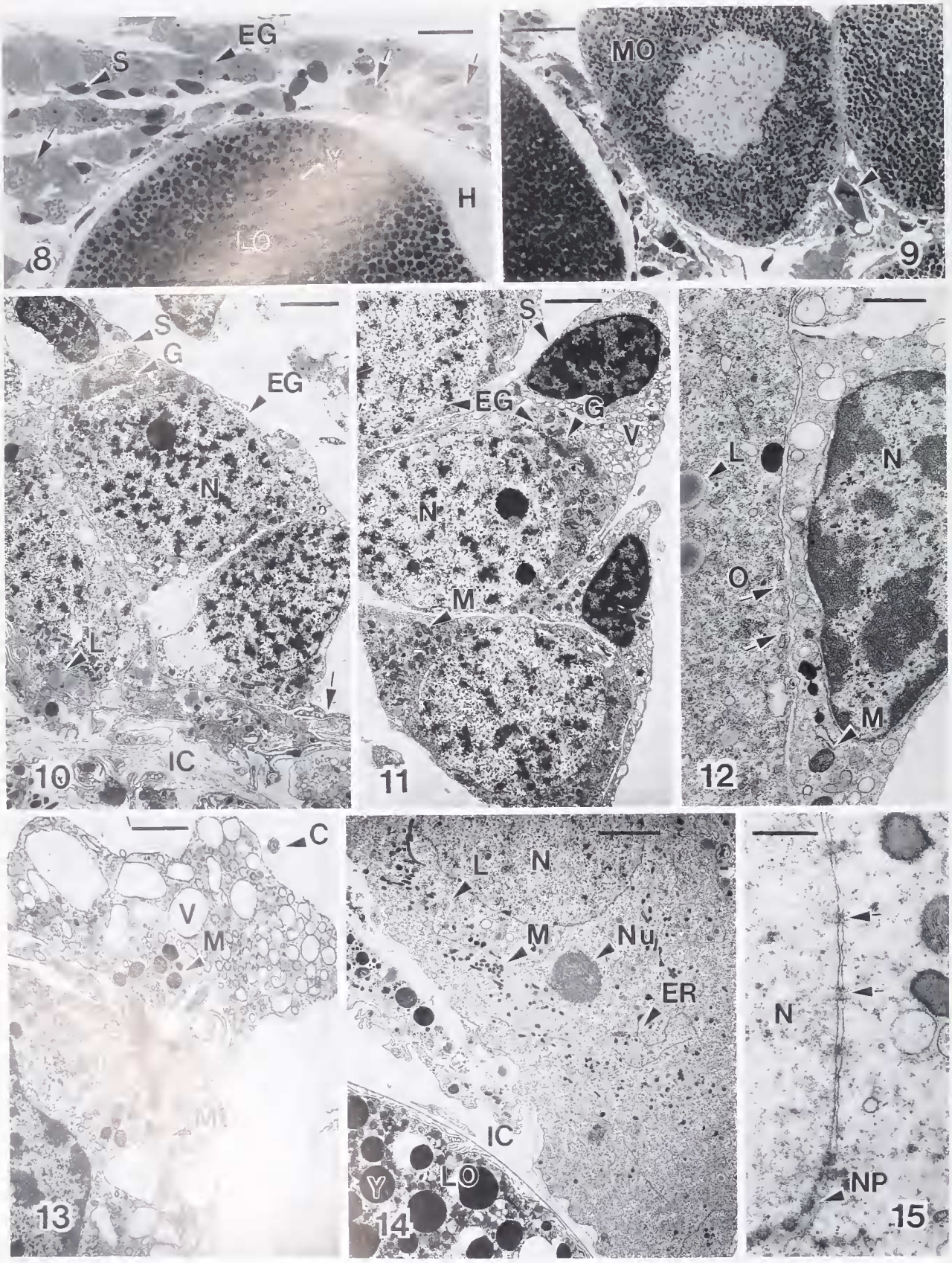
Figure 3. SEM of an ovary (Ov) and peritoneal suspensors (arrow). B, bursal wall. Scale = 100 μm.

Figure 4. Light microscopic (LM) section of an ovary with late (LO), mid- (MO), and previtellogenic (PO) oocytes. EG, early germinal cells, H, haemal fluid. Scale = 75 μm.

Figure 5. Transmission electron micrograph (TEM) of the ovary wall in cross section. The inner and outer sacs are separated by the genital coelom (GC). A, amoebocyte, BL, basal lamina, Ep, coelomic epithelium, F, collagen fibrils, G, granulated process, IC, inner connective tissue, J, cell junction, M, myoepithelial cell, OC, outer connective tissue, P, visceral peritoneum, Arrow, basal process of the primary oocyte (O). Scale = 2.0 μm.

Figure 6. Next to a vitellogenic oocyte (O) the inner connective tissue layer is filled with haemal fluid (H). The inner basal lamina (BL) is thin while the outer basal lamina is thick and plicated. CM circular muscle cell, F, fibril, GC, genital coelom, LM, longitudinal muscle cell. Scale = 1.5 μm.

Figure 7. The inner connective tissue (IC) is devoid of haemal fluid next to relict oocyte material (R). A, amoebocyte, BL, basal lamina, Ep, epithelium, G, granulated process, GC, genital coelom, M, muscle, N, nerves, OC, outer connective tissue, P, peritoneum, S, somatic cell. Scale = 3.0 μm.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end of oogenesis, are 400 μm in diameter (Hendler, 1979). Oviducts are absent and the oocytes are spawned into the bursal sac one to a few at a time by an unknown mechanism. Subsequent fertilization presumably occurs in the bursa. The embryos develop in the bursa leaving the adult at the two to three arm segment stage (Fig. 2).

The ovary consists of two parts, the inner and outer sac, which are separated by the genital coelom (Figs. 5, 7, 45). Each sac is composed of three layers of tissue. The outer sac consists of the visceral peritoneum, a central connective tissue layer, and the genital coelomic epithelium. The inner sac is composed of the internal coelomic epithelium, a connective tissue layer that contains the haemal sinus, and the germinal epithelium.

The outer covering of the ovary, the visceral peritoneum, is a thin layer of epithelial and myoepithelial cells, each containing an elongate nucleus, mitochondria, vesicles and lysosomes (Figs. 5, 7). Cell bodies of the peritoneal cells were rarely encountered and sparse cilia arising from the peritoneal layer were seen in the light microscopic sections. Small bundles of neurons containing dense-cored (60–120 mm diameter) and clear vesicles (60–140 nm diameter) run beside the myoepithelial cells and are covered by cytoplasmic extensions from these cells (Figs. 7, 35). Processes containing large electron-dense granules (500–1500 nm length) are also present in the peritoneum (Figs. 5, 7). In general, the ovary is well innervated with longitudinally orientated nerve tracts surrounded by epithelial cell processes. Zonulae adherentes and septate junctions join the epithelial cells (Fig. 5), and occasional hemidesmosomes link the peritoneum to the underlying basal lamina.

The outer connective tissue layer, delimited on either side by basal laminae, contains striated collagen fibrils and unstriated fibrils embedded in an interfibrillar matrix (Figs. 5, 7). Amoebocytes and cell processes are occasionally seen in the connective tissue (Fig. 7).

The genital coelom varies in width and often has reduced dimensions due to the presence of large vitellogenic oocytes in the germinal layer. Apart from a few coelomocytes and cell debris (Fig. 5), the coelom appears devoid of contents. In life, however, the coelom is presumably a fluid-filled space. A squamous epithelium lines the coelom and the inner coelomic epithelium includes circular and longitudinal myoepithelial cells (Fig. 6). The circular musculature of the inner sac is the major muscle layer of the ovary. Cytoplasmic contents of the coelomic epithelial cells include an elongate nucleus, ribosomes, vesicles, microtubules, rough endoplasmic reticulum (RER), and Golgi complexes (Figs. 5, 7). It was noted that a few of the cells were ciliated. Nerves are present in the outer and inner coelomic epithelia (Figs. 5, 7, 35), and large granulated processes are a prominent feature of the inner epithelium (Fig. 5). The epithelial cells are joined to the underlying basal lamina by hemidesmosomes.

The inner connective tissue layer is delimited by basal laminae that have a tortuous outline (Figs. 5–7), although the inner basal lamina is straight when juxtaposed with vitellogenic oocytes (Figs. 6, 35, 37). As for the outer sac, the inner connective tissue contains collagen fibrils and unstriated fibrils (Fig. 5). The genital haemal sinus is also a component of the inner connective tissue layer, but is only evident next to vitellogenic oocytes. Haemal fluid accumulates around the vitellogenic oocytes (Fig. 4, 6), but is absent from portions of the connective tissue next to previtellogenic and residual oocytes (Figs. 5, 7). The fluid stains pale purple with Azur II/methylene blue (Figs. 4, 8) and has a flocculent, granular appearance (Figs. 6, 38). Fibrillar elements of the connective tissue are also embedded in the haemal fluid (Figs. 6, 38). Amoebocytes are occasionally encountered in the inner connective tissue (Fig. 5).

The germinal epithelium consists of germinal and so-

Figure 8. LM cross section near the base of the ovary showing a group of early germinal cells (EG), including dividing oogonia (arrows). H, haemal fluid, LO, late vitellogenic oocyte, S, somatic cell, Y, yolk body. Scale = 22 μm.

Figure 9. Mitotic profile (arrow) between midvitellogenic oocytes (MO). Scale = 40 μm.

Figure 10. Early germinal cells (EG) lying alongside and *not* in an evagination of the inner connective tissue layer (IC). G, Golgi complex, L, lipid droplet, N, nucleus, S, somatic cell. Arrow, basal process. Scale = 4.0 μm.

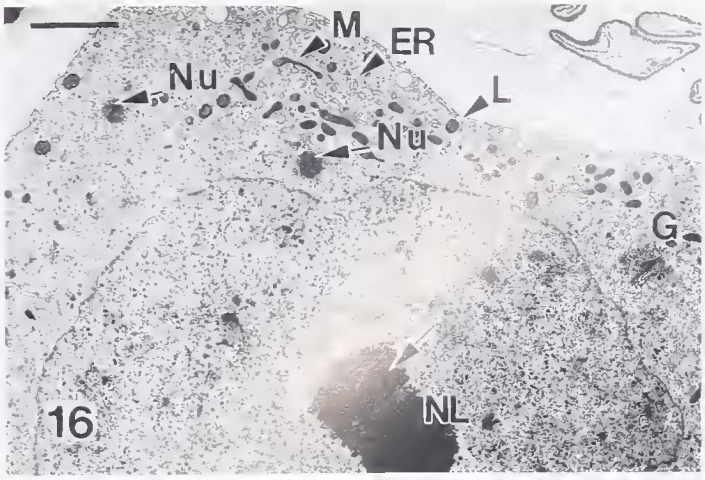
Figure 11. The somatic cells (S) partially surround the early germinal cells (EG) and have an abundance of vesicles (V) in their cytoplasm. G, Golgi complex, M, mitochondrion, N, nucleus. Scale = 4.0 μm.

Figure 12. Small extrusions of the somatic cell (arrows) are closely applied to the oocyte (O). L, lipid droplet, M, mitochondrion, N, nucleus. Scale = 1.0 μm.

Figure 13. A somatic cell not associated with an oocyte. C, cilium, G, Golgi complex, M, mitochondrion, Mt, microtubule, V, vesicles. Scale = 1.5 μm.

Figure 14. Previtellogenic oocyte in a depression of the inner connective tissue layer (IC). ER, endoplasmic reticulum, L, lipid droplet, LO, late vitellogenic oocyte, M, mitochondria, N, nucleus, Nu, nuage, Y, yolk body. Scale = 5.0 μm.

Figure 15. Nucleocytoplasmic exchange is indicated by the basophilic material (arrows) emerging from the nuclear pores (NP). N, nucleus. Scale = 1.0 μm.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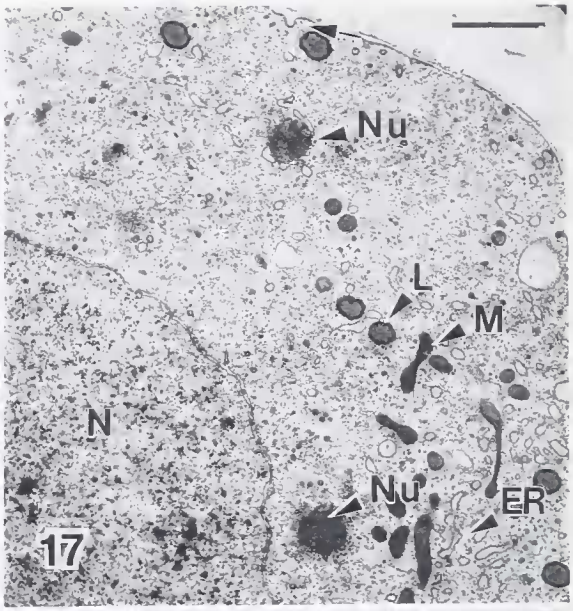
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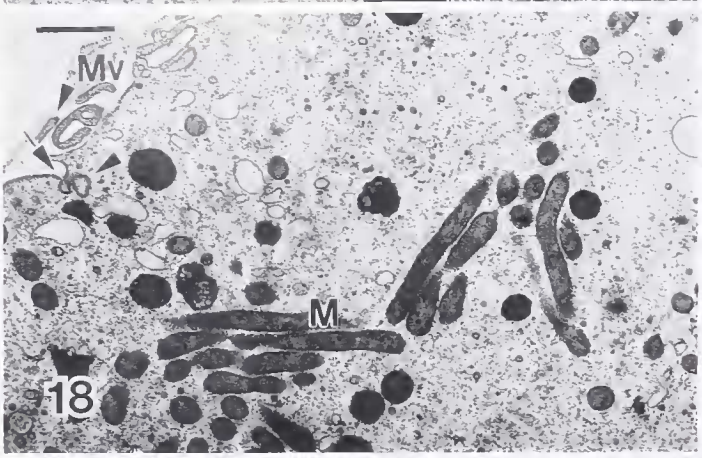
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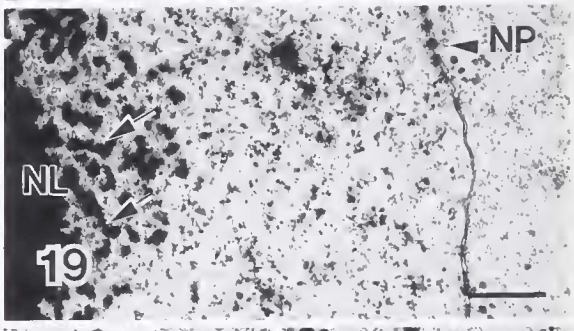
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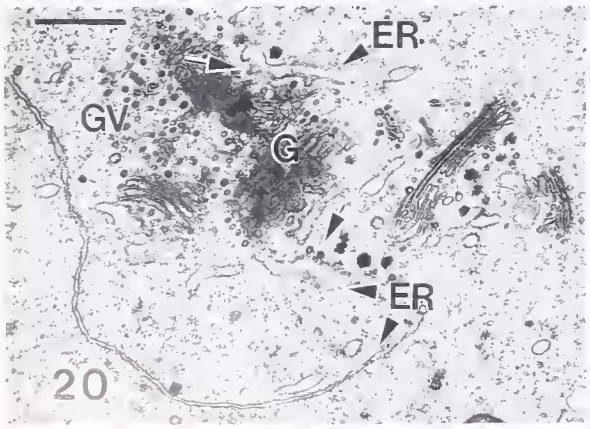
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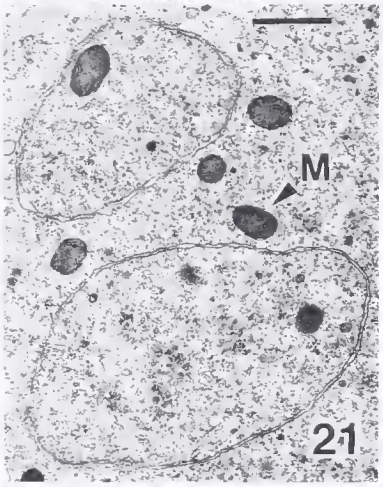
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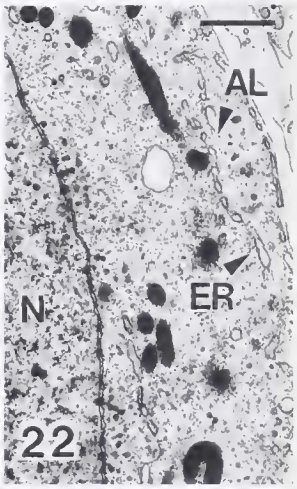
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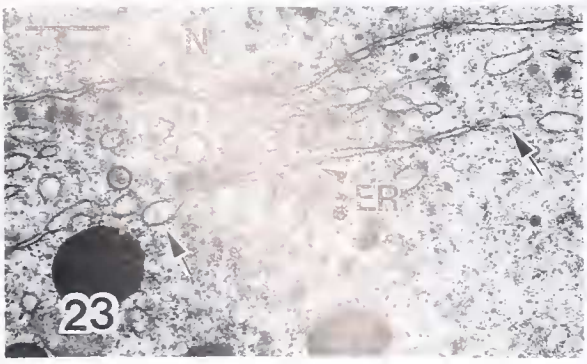
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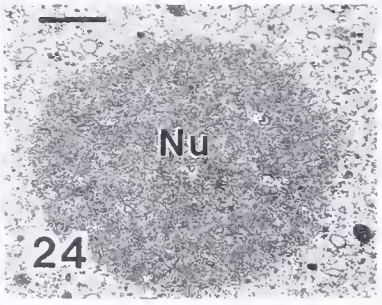
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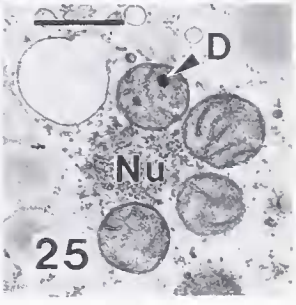
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matic cells that follow the contour of the inner basal lamina (Figs. 5, 10). The ovary has a poorly organized appearance because the oogenic stages are not spatially segregated (Fig. 4). In addition, the attachment of the developing oocytes with the germinal epithelium becomes reduced to a short stalk (Fig. 26).

Groups of small germinal cells (15–25 μm in length) are present at the base of the ovary near its attachment to the genital plate (Figs. 4, 8). The small germinal cells include proliferating oogonia in various stages of mitosis and early primary oocytes (Fig. 8). Oogonial proliferation occurs elsewhere in the ovary and mitotic profiles were also seen in cells adjacent to vitellogenic oocytes (Fig. 9). The germinal cells with few or no nuclear pores may be oogonia (Fig. 10), based on the criteria used in other studies (Piatigorsky, 1975; Kanatani and Nagahama, 1983). Characteristic features of the small germinal cells include a large nucleus (8–15 μm diameter) and a small amount of cytoplasm (Figs. 10, 11). The nucleus contains one or two nucleoli and patches of clumped chromatin. Cytoplasmic organelles include numerous free ribosomes, groups of mitochondria, vesicles, Golgi complexes and occasional lipid droplets (Figs. 10–12).

The somatic cells (8–17 μm long) of the germinal epithelium are squamous, irregularly shaped, and ciliated (Figs. 10–13). They are distinguished from the germinal cells by their small, dark nucleus (6–11 μm length) with chromatin lining the nuclear envelope (Fig. 12). Somatic cells also contain an abundance of electron-lucent vesicles, mitochondria, a Golgi complex, microtubules, lipid droplets, and relict oocyte material (Figs. 10–13). Cytoplasmic extensions of the somatic cells interdigitate with each other and form an incomplete follicle around the germinal cells (Figs. 10, 11). Small extrusions from the somatic cells are closely applied to the oocyte surface, but junctional complexes were not observed (Fig. 12). The somatic cells do not appear to be involved in oocyte nutrition and their association with the oocytes terminates early in oogenesis.

Previtellogenic oocytes

Early previtellogenic oocytes (30–50 μm diameter) are attached to the basal lamina or to neighboring cells by a basal process (Figs. 5, 10). As the oocytes grow, they press against the ovary wall forming a depression in the inner connective tissue (Figs. 14, 45). Previtellogenic oocytes have a prominent germinal vesicle containing a single nucleolus and dispersed chromatin (Fig. 16). During the previtellogenic stage there is an increase in the size of the germinal vesicle and in the amount of cytoplasm. The nucleolus has two regions, an electron-dense core and a peripheral nucleolonema that is granular and emanates chromatin-like strands (Figs. 16, 19). The nuclear envelope is perforated by annulated pores that appear to be a site for nucleocytoplasmic exchange, indicated by the basophilic material emerging from the pores (Figs. 15, 19).

Early previtellogenic oocytes contain free ribosomes, mitochondria, lipid droplets, microtubules, Golgi complexes, and some ER (Figs. 16–18). Mitochondriogenesis occurs in the previtellogenic stage, with an abundance of pleomorphic mitochondria that are often concentrated in a 'mitochondrial cloud' (Fig. 18). Many of the mitochondria have an eccentrically placed dense granule (Fig. 25). Golgi complexes located in the perinuclear ooplasm (Fig. 16) increase in number and dominate this region of the oocyte (Fig. 20) and as oogenesis proceeds, the Golgi disperse towards the cortical ooplasm. Each Golgi complex consists of 7–9 cisternae and many of them have a single cisterna of ER positioned next to the forming face (Fig. 20). Golgi vesicles containing electron-dense material arise from the maturing face of the Golgi and the ER also gives rise to electron-dense vesicles (Fig. 20). Elon-

Figures 16–26. Previtellogenic oocytes.

Figure 16. The nucleolus (NL) is bipartite with a peripheral nucleolonema (arrow). ER, endoplasmic reticulum, G, Golgi complex, L, lipid droplet, M, mitochondrion, Nu, nuage. Scale = 3.0 μm.

Figure 17. The nuage (Nu) is surrounded by endoplasmic reticulum (ER) or is independent. L, lipid droplet, M, mitochondrion, N, nucleus. Arrow, endocytotic profile. Scale = 2.0 μm.

Figure 18. A coated pit (arrow) and coated vesicle (arrowhead) in a previtellogenic oocyte. M, mitochondrial cloud, Mv, microvillus. Scale = 1.0 μm.

Figure 19. Portion of the nucleolus (NL) with peripheral strands (arrows). The nuclear envelope is partially perforated by pores (NP). Scale = 1.0 μm.

Figure 20. Perinuclear Golgi complexes (G) and associated endoplasmic reticulum (ER). The ER gives rise to transport vesicles (arrows). Note the long ER cisterna. GV, Golgi vesicles. Scale = 1.0 μm.

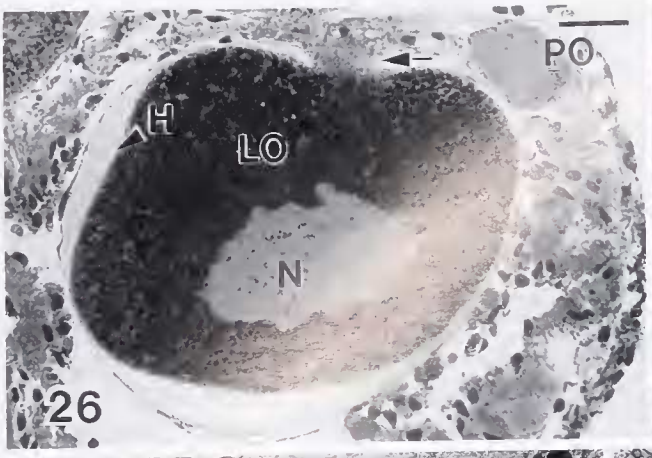
Figure 21. Circular endoplasmic reticulum, M, mitochondrion. Scale = 1.0 μm.

Figure 22. Single profile of annulate lamellae (AL) and associated endoplasmic reticulum (ER). N, germinal vesicle. Scale = 1.0 μm.

Figure 23. ER cisterna with dilated tips (arrows). N, nucleus. Scale = 0.5 μm.

Figure 24. The large nuage (Nu) seen in Fig. 14. Scale = 1.0 μm.

Figure 25. Small nuage (Nu) surrounded by mitochondria. D, dense granule. Scale = 1.0 μm.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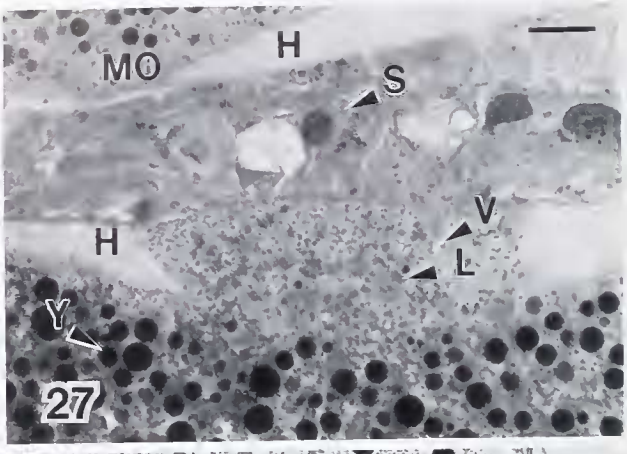
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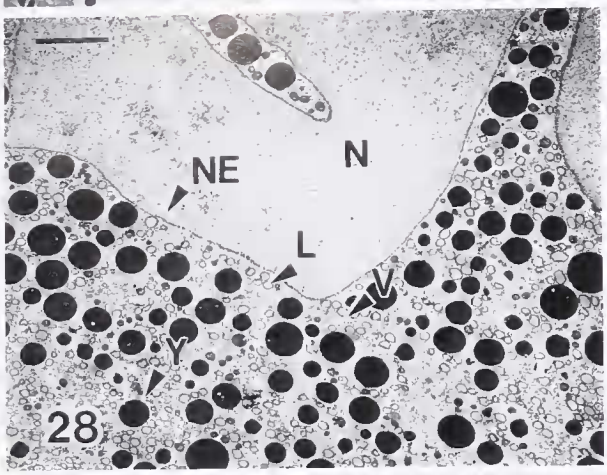
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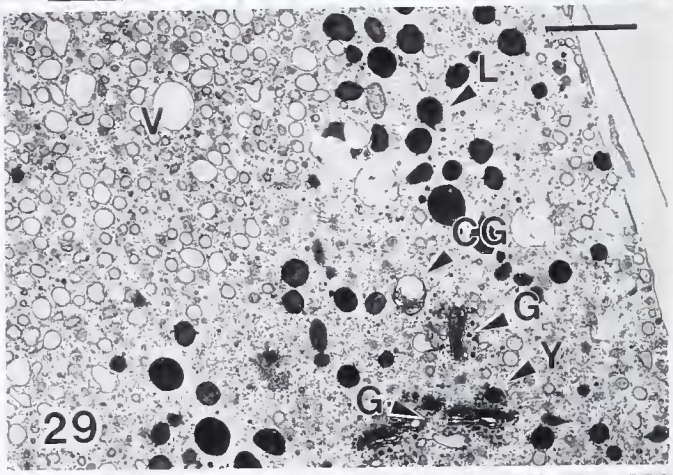
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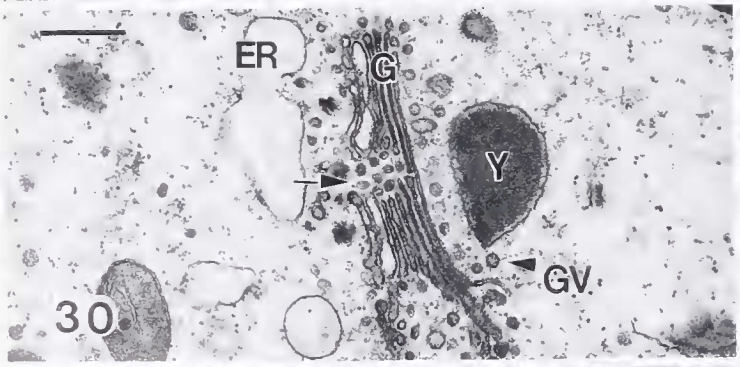
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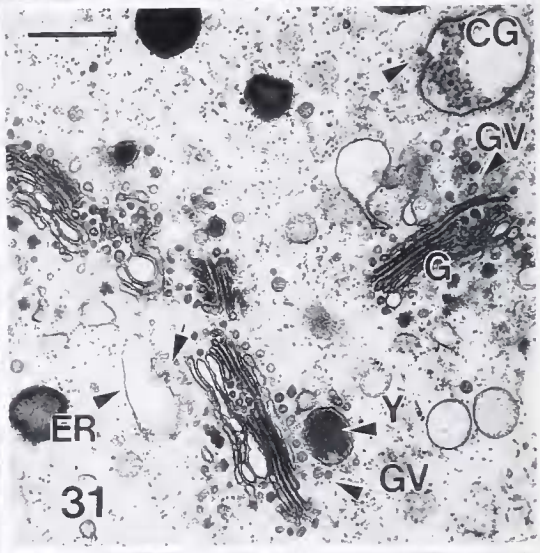
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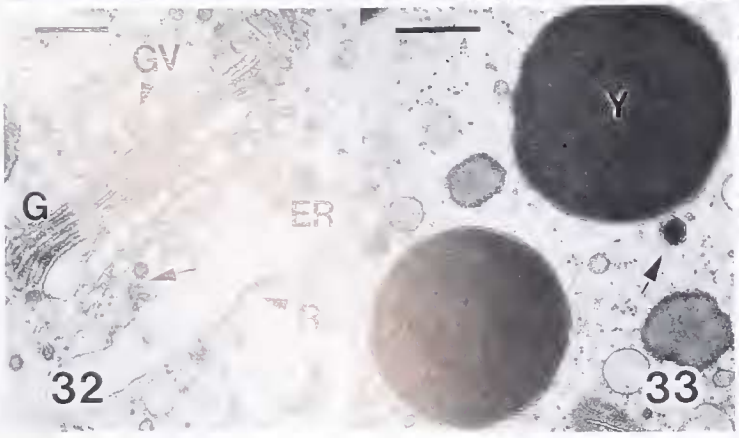
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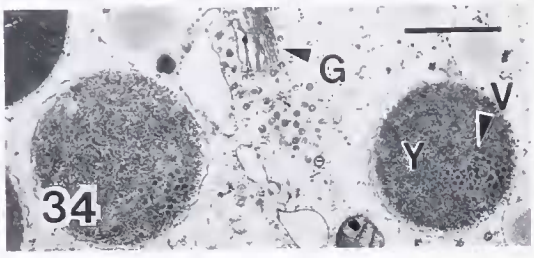
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gate and circular ER are scattered in the cytoplasm (Figs. 14, 20, 21). All the ER in the early oocytes appears to lack attached ribosomes (Figs. 20, 21). Dilated tips of the ER cisternae give rise to small vesicles (Fig. 23) and appear to form single profiles of annulate lamellae (Fig. 22). Evidence of ER vesiculation is present throughout previtellogenesis and membranous, electron-lucent vesicles are a prominent feature of late previtellogenic and early vitellogenic oocytes (Fig. 29). The function of these vesicles is not known.

Fibrogranular material that lacks a unit membrane, similar to the nuage seen in other oocytes (Eddy, 1975; Wourms, 1987), is common in previtellogenic oocytes (Figs. 14, 17). This material, possibly derived from nucleocytoplasmic transfer, is present as large (4–5 μm diameter, Figs. 14, 24) and small (0.3–1.0 μm diameter, Figs. 17, 25) fibrogranular bodies (FGBs). The small FGB's are often surrounded by mitochondria or ER cisternae (Figs. 17, 25). During oogenesis the FGB's disperse.

The number of lipid droplets (0.3–0.8 μm diameter) increases throughout oogenesis and they appear to arise *de novo* in the cytoplasm. Lipid droplets are not membrane-bound and are dispersed throughout the ooplasm (Fig. 28). They are not associated with other organelles and it is unknown how they originate.

Microvilli are elaborated in local patches along the oolemma of late previtellogenic oocytes and endocytosis is evident with coated pits (150–200 μm) present at the bases of the microvilli (Fig. 18). Endocytosis also occurs in early previtellogenic oocytes that lack microvilli (Fig. 17).

Vitellogenic oocytes

The onset of the vitellogenic period is marked by the presence of yolk bodies in the ooplasm, starting with oocytes approximately 100 μm in diameter (Figs. 4, 26). As the oocytes increase in size, they bulge outwards, causing the inner basal lamina to unfold and follow the contours of the oocyte (Figs. 35, 37, 45). They become increasingly independent of the germinal layer with each vitellogenic oocyte residing in a sac-like depression of the inner connective tissue and attached to the germinal epithelium by a neck-like stalk (Figs. 26, 27). The attachment region contains lipid droplets, electron-lucent vesicles, and is usually devoid of yolk bodies (Fig. 27). Continued oocyte growth causes attenuation of the inner basal lamina, whereas the outer basal lamina is thicker and plicated (Figs. 6, 35). The presence of an attachment stalk in advanced oocytes suggests that the oocytes retain their association with the germinal epithelium throughout vitellogenesis.

Haemal fluid accumulates in the connective tissue layer adjacent to vitellogenic oocytes (Figs. 4, 26), starting in early vitellogenesis. Although each oocyte is surrounded by haemal fluid, it remains separated from the fluid by the inner basal lamina (Figs. 35, 37). The association between the genital haemal sinus and the oocytes is particularly evident during late and mid-vitellogenesis when haemal fluid accumulates in depressions of the oocytes and on either side of the attachment region (Figs. 4, 26, 27). However, haemal fluid is not restricted to these areas. Serial sections of the ovary revealed that the haemal investment around each oocyte is not uniform

Figures 26–34. Vitellogenic oocytes.

Figure 26. A late vitellogenic oocyte (LO) in a sac-like evagination of the inner connective tissue layer, surrounded by haemal fluid (H) and attached to the germinal layer by a short stalk (arrow). N, germinal vesicle, PO, previtellogenic oocyte. Scale = 50 μm.

Figure 27. Stalk connecting the oocyte to the germinal layer. H, haemal fluid, L, lipid droplet, MO, midvitellogenic oocyte, S, somatic cell, V, vesicle, Y, yolk body. Scale = 9.5 μm.

Figure 28. The nuclear envelope (NE) is convoluted and the germinal vesicle (N) appears amorphous. L, lipid droplet, V, vesicle, Y, yolk body. Scale = 5.0 μm.

Figure 29. Early vitellogenic oocyte with the cytoplasm dominated by electron-lucent vesicles (V). One Golgi complex (G) is beside a small yolk body (Y) and the other beside a cortical granule (CG). See Figure 31. L, lipid droplet. Scale = 3.0 μm.

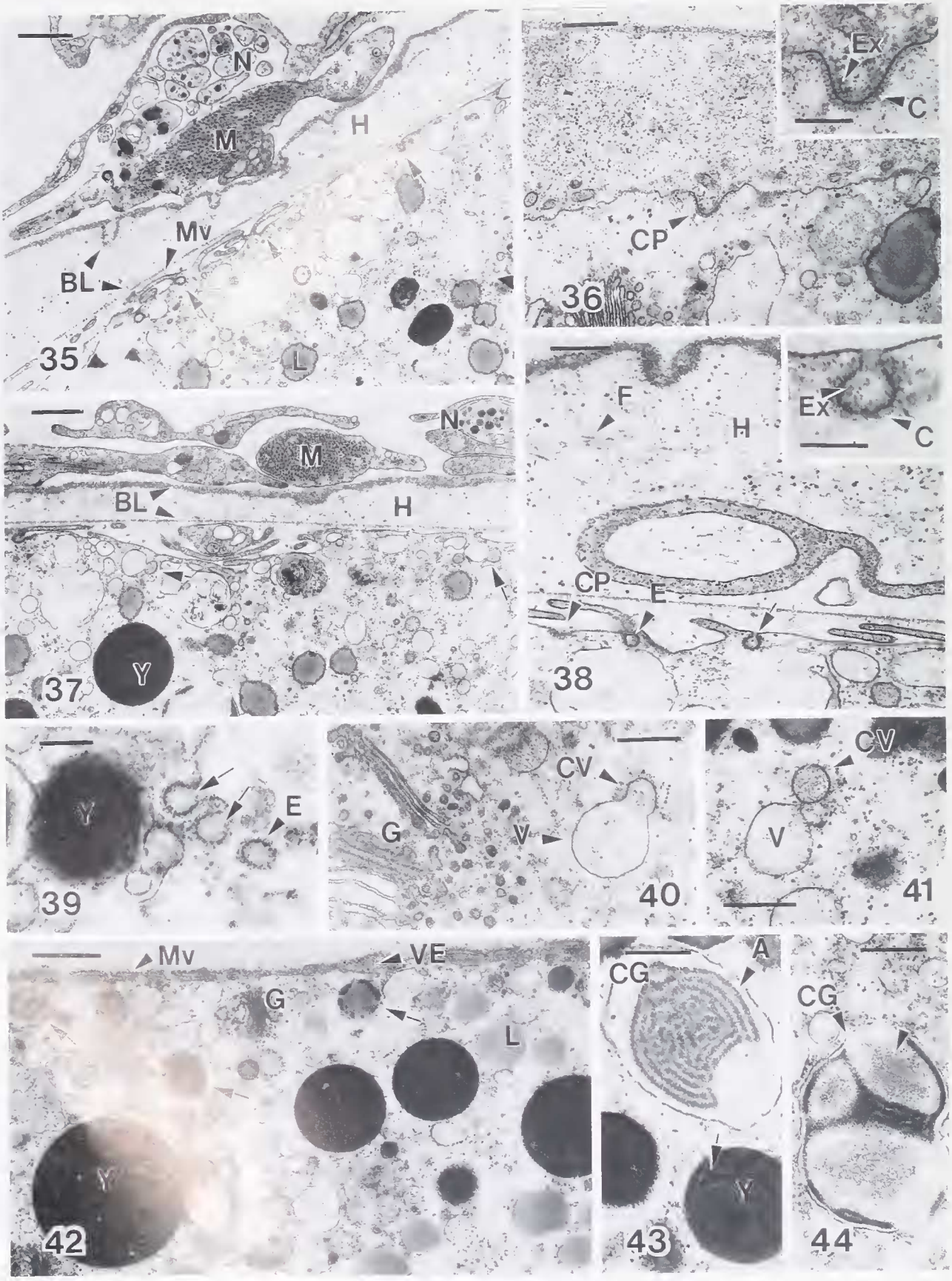
Figure 30. Yolk body (Y) formation in an early vitellogenic oocyte. Transport vesicles (arrow) derived from the endoplasmic reticulum (ER) appear to fuse with the forming face of the Golgi complex (G), which also gives rise to vesicles (GV). Scale = 0.5 μm.

Figure 31. Golgi (G) region seen in Fig. 29. ER-projections (arrow) give rise to vesicles and Golgi vesicles (GV) appear to fuse with the yolk body (Y) and with (arrowhead) the cortical granule (CG). Scale = 0.75 μm.

Figure 32. Portion of the endoplasmic reticulum (ER) with attached ribosomes (R) in a midvitellogenic oocyte. ER-projections (arrow) form transport vesicles that appear to fuse with the forming face of the Golgi (G). GV, Golgi vesicles. Scale = 0.3 μm.

Figure 33. Dense yolk body (Y) and attached vesicle (arrow) in a midvitellogenic oocyte. Scale = 0.6 μm.

Figure 34. Granular yolk body (Y) with vesicular inclusions (V) in a midvitellogenic oocyte. G, Golgi complex. Scale = 1.0 μm.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and that the fluid does not appear to exhibit an affinity for any particular region of the oocytes.

The space between the oolemma and the inner basal lamina appears devoid of contents in oocytes fixed with seawater buffered glutaraldehyde (Figs. 35, 37). However, with the cacodylate method flocculent material reminiscent of haemal fluid is occasionally present in this space (Fig. 36).

During vitellogenesis, the volumes of the ooplasm and the germinal vesicle continue to increase (Figs. 26, 28). Early vitellogenic oocytes contain small yolk bodies (0.6–1.6 μm diameter), lipid droplets, mitochondria, cortical granules, electron-lucent vesicles, nuage, Golgi complexes, ER, and free ribosomes; the nuclear envelope is undulated (Figs. 26, 28). Portions of the ooplasm dominated by the membranous vesicles encountered in the previtellogenic stage are markedly devoid of yolk bodies (Fig. 29). These vesicles subsequently disperse through the ooplasm (Fig. 28). In midvitellogenic oocytes, Golgi complexes abound in the cortical ooplasm (Fig. 29). As in previtellogenic oocytes, most of the ER appears to be smooth (Figs. 30, 31), however patches of ER with attached ribosome-like particles were observed in cisternae associated with a Golgi complex (Fig. 32). These patches of rough ER on otherwise smooth cisternae are rare and only evident in vitellogenic oocytes. In vitellogenic oocytes, ER is present only as short cisternae adjacent to a Golgi complex.

In early vitellogenic oocytes, yolk formation occurs in the perinuclear ooplasm, whereas in late vitellogenic oocytes yolk formation is largely cortical. Newly formed yolk bodies (0.14–0.80 μm length) are positioned near a Golgi complex (Figs. 30, 31). They have an electron-dense or granular core surrounded by an electron-lucent, halo-like cortex. It appears that the Golgi complex and the ER are involved in the elaboration of the yolk bodies. The process starts with small expansions of the ER cisternae that project towards the Golgi complex (Figs. 31, 32). These projections often have an external coat and their detachment gives rise to transport vesicles that are coated. A flocculent material is contained within the vesicles and they appear to fuse with the forming face of the Golgi (Fig. 30). Vesicles containing material of variable electron density are released from the maturing face of the Golgi and fuse to form yolk bodies (Figs. 30–32). During the vitellogenic stage, the yolk bodies continue to grow through successive incorporation of material contained within Golgi vesicles (Figs. 30, 31, 33). Midvitellogenic oocytes contain yolk bodies (0.7–2.5 μm diameter) of variable electron density. Growing yolk bodies often have a light halo and may contain membranous strands (Fig. 43). Some yolk bodies are light and granular with vesicular inclusions (Fig. 34), whereas others are dark and homogeneous (Fig. 33). As vitellogenesis proceeds, the yolk material condenses and the cytoplasm of advanced oocytes (300–400 μm diameter) is dominated by yolk bodies that are uniformly electron-dense and 2–5 μm in diameter (Fig. 28).

The oolemma changes throughout vitellogenesis with the continued elaboration of microvilli, which have a scattered distribution around the oocyte. Endocytotic activity is present throughout vitellogenesis and is particularly conspicuous at the bases of the microvilli with up to three coated pits per μm of oolemma (Figs. 35–39). The cytoplasmic surface of the pits has a fuzzy or spiked appearance and flocculent material adheres to the extracellular surface (Figs. 36, 38, 39). The pit and adherent material appear to invaginate to form a cup-like depres-

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Figures 35–44. Late vitellogenic oocytes.

Figure 35. Endocytotic activity along the oolemma (arrows). BL, basal lamina, H, haemal fluid, L, lipid droplet, M, muscle, Mv, microvillus, N, nerve. Scale = 1.0 μm.

Figure 36. The cup-shaped coated pit (CP) has a spiked cytoplasmic surface (C, insert) and flocculent material which surrounds the oocyte adheres to the extracellular surface of the coated pit (Ex, insert). The fixative was buffered with cacodylate. Scale = 1.0 μm. Insert scale = 0.2 μm.

Figure 37. Endosomes appear to fuse with smooth vesicles in the cortical ooplasm (arrows). BL, basal lamina. H, haemal fluid, M, muscle, N, nerve, Y, yolk body. Scale = 1.0 μm.

Figure 38. Formation of an endosome. A coated pit (CP) is invaginated to form an omega-shaped pit (arrow and insert) and internalized to form a coated vesicle. The cytoplasmic side (C) of the pit has a fuzzy appearance and flocculent material on the extracellular surface (Ex) is being internalized (insert). The fixative was buffered with seawater. F, collagen fibril, H, haemal fluid. Scale = 0.5 μm. Insert scale = 0.15 μm.

Figure 39. Three endocytotic profiles; two omega figures (arrows) and an endosome (E), Y, yolk droplet. Scale = 0.2 μm.

Figures 40–41. Coated (CV) and smooth vesicles (V) fusing in the Golgi (G) area. Scale = 0.5 μm.

Figure 42. Peripheral ooplasm of an oocyte fixed with the cacodylate method. The cortical granules (arrows) have contrasting dark and light areas. G, Golgi complex, L, lipid droplet, Mv, microvillus, VE, vitelline envelope, Y, yolk body. Scale = 1.5 μm.

Figures 43–44. Cortical granules (CG) of oocytes fixed with the seawater method with annular (A) and amorphous (arrowhead) contents. Arrow, membranous strand in the yolk (Y) body. Scale 0.5 μm.

sion or an omega figure (Figs. 36, 38, 39) and are then internalized, forming a coated vesicle (endosome). Newly formed endosomes are common near the oolemma (Figs. 37, 39). In Figure 37, the flocculent material surrounding the oocyte is present in the pit and appears to be incorporated by the oocyte. The endosomes appear to lose their cytoplasmic coat soon after internalization and are indistinguishable from other smooth vesicles that abound in the cortical ooplasm. There is evidence that the coated vesicles fuse with smooth vesicles near the oolemma (Fig. 37). Coated vesicles fusing with smooth vesicles are also present in the Golgi region (Figs. 40, 41), but it is not known if they are derived from coated pits. The fate of the material internalized through endocytosis could not be followed.

Cortical granule formation is initiated in the early vitellogenic stage. In these oocytes, cortical granules are found in the endoplasm and in the cortical ooplasm. Late vitellogenic oocytes have abundant cortical granules (1.4–1.6 μm diameter) in the peripheral ooplasm (Fig. 42). The cortical granules are membrane-bound, irregular in shape, and contain material with differing staining densities (Figs. 42–44). Their appearance is influenced by the method of fixation. In material preserved with cacodylate-buffered glutaraldehyde, the granules have contrasting dark and light regions and contain a flocculent material (Fig. 42). However, in oocytes fixed with the seawater method, the cortical granules are less electron-dense and the contents have an annular or flocculent appearance (Figs. 43, 44). Some of the cortical granule material may originate from ER-Golgi activity. Golgi-derived vesicles appear to coalesce with the cortical granules adjacent to the oolemma (Fig. 31).

A vitelline envelope is evident in advanced oocytes fixed with the cacodylate method (Fig. 42). The envelope consists of dark fibrous material closely applied to the oolemma, ranging in diameter from 60 to 300 nm. Additional extracellular coats, including a jelly layer, were not observed but may be deposited in the post-vitellogenic stage in the ovarian lumen prior to spawning.

Discussion

The ovary wall

The ovary of *O. paucispina* is morphologically similar to that of other ophiuroids and to the ovaries of asteroids and echinoids. It also have a two-sac structure (Davis, 1971; Walker, 1979, 1982). Because the ovary is continually expanded with advanced oocytes, the epithelial layers of the ovary have an attenuated profile. Flagellated-collar cells present in the peritoneum of asteroid ovaries (Walker, 1979) are not present in *O. paucispina*, and, in general, there appears to be a paucity of ciliated cells in the ovarian peritoneum. Suspensor cells, similar to those seen in the ovary, also connect with the testes of *O. paucispina* and with the testes of *Amphipholis squamata* (Buckland-Nicks *et al.*, 1984; pers. obs.). The epithelial layers of the ovary of *O. paucispina* contain myoepithelial cells, a characteristic of echinoderm coelomic epithelia (Rieger and Lombardi, 1987). By contrast, Davis (1971) did not report the presence of musculature in the ovarian coelomic epithelia of *Ophioderma panamense* Lütken and *Ophiopteris papillosa* (Lyman). Contraction of the ovarian musculature presumably assists spawning, which may occur through rupture of the ovary wall, as suggested for other ophiuroids that lack an oviduct (Davis, 1971).

The ovary of *Ophiolepis paucispina* is well innervated by neurons that run beside the myoepithelial cells. These neurons contain vesicles characteristic of neurons associated with echinoderm muscles (Cobb, 1987), and may innervate the ovarian musculature. Large granulated processes are common in the inner coelomic epithelium and are similar to the neurosecretory-like cells common in echinoderm epithelia (Cobb, 1987). The function of these processes is not known, but their structure and position indicates that they may serve an endocrine function in oocyte development, as suggested for asteroid, crinoid, and holothuroid species (Holland, 1971; Ferrand, 1983; Smiley and Cloney, 1985). The connective tissue layers of the ovary of *O. paucispina* are primarily supportive and the inner connective tissue contains the genital haemal sinus which surrounds vitellogenic oocytes.

Germinal layer and the role of somatic cells

Because oocytes at all stages of development are interspersed in the ovary of *Ophiolepis paucispina*, a distinct germinal layer is not apparent. Oogonial proliferation occurs near the base of the ovary, similar to *Ophioderma brevispinum* (Say) and *Ophiura albida* Forbes (Tyler, 1976; Hendler and Tyler, 1986), but also takes place elsewhere in the ovary, suggesting that there may not be a discrete proliferative region. For *O. paucispina* it is not known where the primordial germ cells originate. In *Gorgonocephalus eucnemis* (Müller and Troschel), the primordial germ cells enter the ovary through haemal channels and oocyte proliferation occurs in the genital haemal sinus (Patent, 1976). Similarly, the primordial germ cells of a holothuroid species are located in the connective tissue at the base of the ovary (Smiley, 1988).

Oogenesis in echinoderms may be solitary or follicular (Walker, 1982), and, although the small germinal cells and early oocytes of *Ophiolepis paucispina* are covered by epithelial cells, oogenesis is solitary. The somatic cells appear to provide structural support for the early oocytes

and may contribute to the microenvironment required for early development. In the vitellogenic stage, the somatic cells along the attachment site also appear to play a supportive role. The oocytes of *Ophioderma brevispinum* are also surrounded by follicle cells at an early stage and their association with the germinal layer during vitellogenesis appears to be limited to a short stalk (Hendler and Tyler, 1986). In contrast, the oocytes of *Gorgonocephalus eucnemis* are surrounded by an incomplete follicle throughout their development and attached nurse cells appear to be involved with oocyte nutrition (Patent, 1968). Crinoid oogenesis is also solitary and is strikingly similar to that seen in *O. paucispina*. In several crinoid species the oocytes arise among follicle cells, but oogenesis proceeds with each oocyte in an evagination of the haemal sinus and attached to the germinal layer by a short stalk (Holland *et al.*, 1975; Mladenov, 1986). Accessory cells free in the genital haemal sinus may be involved in oocyte nutrition (Holland and Kubota, 1975).

For the other echinoderm classes, oogenesis appears to be follicular. In asteroids the oocytes are held within a follicle throughout their development (Schroeder *et al.*, 1979; Kanatani and Nagahama, 1983; Beijnink *et al.*, 1984c) and the follicle cells are a potential source of nutrients for oogenesis (Chia, 1968; Beijnink *et al.*, 1984a). Echinoid oocytes are surrounded by accessory cells that provide nutrients for oocyte growth (Walker, 1982; Kanatani and Nagahama, 1983). In holothuroids where oogenesis of one species has been examined, the oocytes develop within a follicle (Smiley and Cloney, 1985; Smiley, 1988).

Oogenesis

Many of the cellular events that occur in the early stages of oogenesis in *Ophiolepis paucispina* are characteristic of the previtellogenic oocytes of echinoderms and other animal groups (Norrevang, 1968; Anderson, 1974; Piatigorsky, 1975; Kanatani and Naganama, 1983; Eckelbarger, 1984; Wourms, 1987). Comparative information on oocyte ultrastructure is available for several ophiuroids (Kessel, 1968; Patent, 1968; Davis, 1971; Bell, 1974; Tyler, 1976). During previtellogenesis of *O. paucispina, Ophioderma panamense,* and *Ophiura albida,* the nucleolus appears to 'spin-out' to form a nucleolonema (Kessel, 1968; Tyler, 1976), a structure thought to be involved in nucleocytoplasmic transport of ribosomes (Kessell, 1968). Nuage deposits similar to those seen in *O. paucispina* are present in the oocytes of several echinoderms, and may be germ cell determinants useful for the identification of germ line cells (Eddy, 1975; Wourms, 1987; Smiley, 1988).

Single profiles of annulate lamellae, similar to those seen in *Ophiolepis paucispina* oocytes, are present in *Acrocnida brachiata* (Montagu) oocytes (Bell, 1974), but differ from the parallel stacks of lamellae seen in the oocytes of *Ophioderma panamense* and those of several holothuroid, crinoid, and echinoid species (Kessel, 1966, 1968; Holland, 1971; Piatigorsky, 1975). Membranous vesicles are a prominent feature of the previtellogenic and early vitellogenic oocytes of *O. paucispina* and appear to be at least partially derived from ER vesiculation. Some of the vesicles may be empty pigment granules, as suggested for the oocytes of an echinoid (Anderson, 1974). The oocytes of *O. paucispina* have a conspicuous pink color in life, but pigment bodies are not evident upon ultrastructural examination. A similar extensive system of vesicles in the oocytes of *O. panamense* gives rise to stacks of annulate lamellae (Kessel, 1968).

Compared with the oocytes of several echinoid, holothuroid, and crinoid species, the oocytes of *Ophiolepis paucispina* have a relatively small amount of ER, and, moreover, the ER in these other oocytes is clearly rough surfaced (Kessel, 1966; Verhey and Moyer, 1967; Bal *et al.*, 1968; Holland, 1971; Aisenshtadt and Vassetzky, 1986). Kessel (1968) notes that the number of ribosomes associated with ER is low in *Ophioderma panamense* oocytes, and describes small patches of rough ER, similar to those seen in this investigation. The presence of small patches of rough ER suggests that the association between the ribosomes and ER cisternae may be transient (Kessel, 1968). Alternatively, the paucity of rough ER may be an artifact resulting from fixation-induced detachment of ribosomes (Eckelbarger, pers. comm.).

Proteid yolk deposition in *Ophiolepis paucispina* is first evident in the Golgi region and appears to result from a sequence of ER-Golgi activity similar to that described for the oocytes of *Ophioderma panamense* and for those of several echinoderms (Kessel, 1966, 1968; Chatlynne, 1972; Piatiagorsky, 1975; Ferrand, 1984; Aisenshtadt and Vassetzky, 1986). In vitellogenic oocytes, the differential distribution of ER in the Golgi region appears to be a functional relationship for yolk deposition. The whorls of ER seen in the oocytes of other ophiuroids (Kessel, 1968; Bell, 1974, Tyler, 1976), thought to be involved in lipid deposition, were not seen in the oocytes of *O. paucispina.*

The cortical granules of *Ophiolepis paucispina* are similar to those seen in several ophiuroids (Kessel, 1968; Tyler, 1976; Holland, 1979; Yamashita, 1984), and appear to arise through the fusion of Golgi vesicles, as reported for *Ophioderma panamense* (Kessel, 1968) and two echinoid species (Anderson, 1974; Geary, 1978). Cortical granule contents are presumably exocytosed after fertilization, as occurs in *Ophiopholis aculeata* (O. F. Müller) and *Amphipholis kochii* Lütken (Holland, 1979; Yamashita, 1984). The influence of different fixation methods on the appearance of cortical granules is also

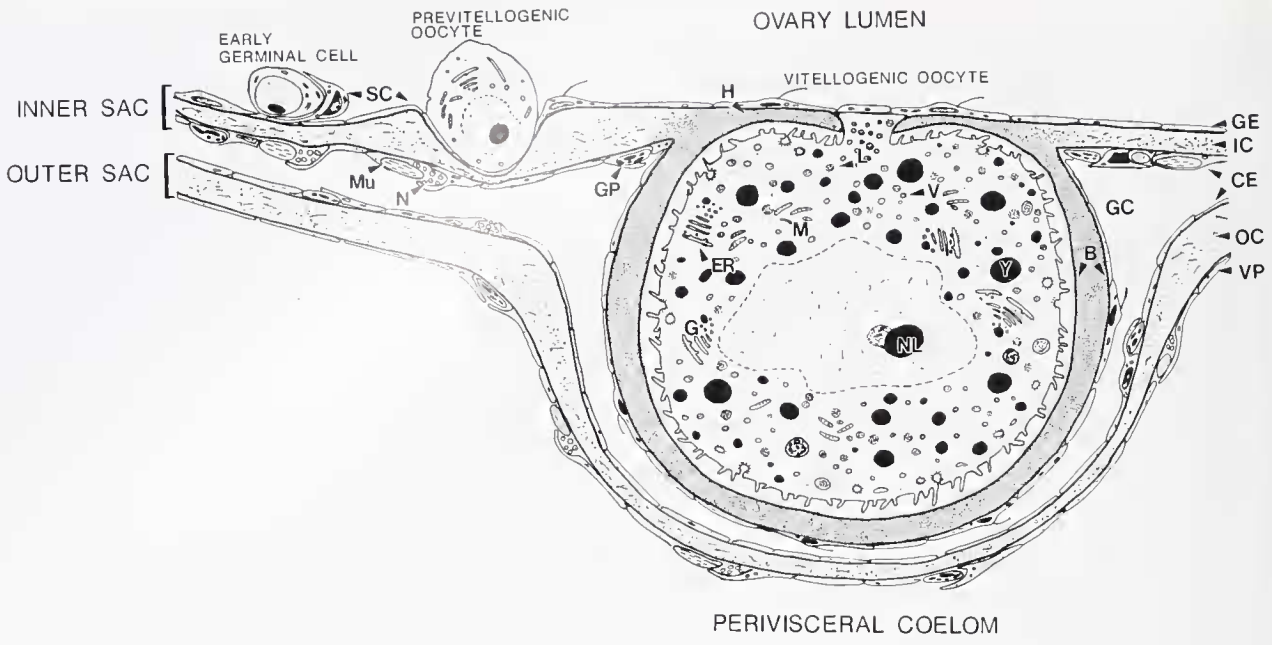

Figure 45. Diagrammatic illustration of a portion of the ovary of *Ophiolepis paucispina* summarizing oogenesis and the formation of the sac-like evagination in the inner connective tissue layer (IC). B, inner and outer basal laminae, ER, endoplasmic reticulum, G, Golgi complex, GC, genital coelom, GE, germinal epithelium, GP, granulated process, H, haemal fluid, L, lipid, M, mitochondria, Mu, muscle, N, nerve, OC, outer connective tissue, SC, somatic cell, VP, visceral peritoneum, Y, yolk body.

noted for the oocytes of several echinoid species (Takashima, 1968). In comparison with *O. aculeata* and *A. kochii* (Holland, 1979; Yamashita, 1984), the vitelline envelope of *O. paucispina* oocytes is thicker. This difference may be influenced by the method of fixation, as an extracellular coat was only preserved with the cacodylate method.

At the termination of oogenesis, the oocytes may ovulate into the ovarian lumen by squeezing through their apical aperture, as described for crinoid oocytes (Holland *et al.*, 1975; Mladenov, 1986).

Haemal sinus and oocyte nutrition

The close contact between the genital haemal sinus and the germinal layer is a characteristic feature of echinoderms (Davis, 1971; Walker, 1982). In *Ophiolepis paucispina* the oocytes bulge outwards as they grow and reside in an individual sac-like depression of the connective tissue layer throughout vitellogenesis (Fig. 45). Subsequently, haemal fluid accumulates around the oocytes. It is unclear whether the oocytes of other ophiuroids also sink into the connective tissue layer. The oocytes of *Gorgonocephalus eucnemis* are bathed in haemal fluid (Patent, 1968), but the details of the relationship between the oocytes and the haemal fluid were not described. In an ultrastructural study of the ovary wall, Davis (1971) reports that the oocytes of *Ophioderma panamense* are surrounded by haemal fluid, whereas those of *Ophiopteris papillosa* and *Ophiothrix spiculata* Le Conte are not. From the information provided (Davis, 1971), the significance of this difference cannot be assessed because oocytes at different stages of oogenesis may have been compared. From the results with *O. paucispina* oocytes, an increase in the volume of the genital haemal fluid would be expected to coincide with the vitellogenic phase. These differences in the relationship between ophiuroid oocytes and the genital haemal sinus warrant further examination through comparative ultrastructural investigations of ophiuroids at all stages of oogenesis.

The relationship between the genital haemal sinus and the developing oocytes of *Ophiolepis paucispina* is similar to that described for several crinoid species (Davis, 1971; Holland, 1971; Holland *et al.*, 1975). Crinoid oocytes also develop in a sac-like evagination and are separated from the surrounding haemal fluid by a basal lamina (Davis, 1971; Holland, 1971; Holland *et al.*, 1975). By contrast, asteroid oocytes are not surrounded by haemal fluid, but develop a close association with the haemal sinus along their basal stalk (Beijnink *et al.*, 1984c). In addition, the volume of the genital haemal sinus of asteroids increases, coincident with the oogenic cycle

(Chia, 1968; Walker, 1974). Holothuroid and echinoid oocytes are also juxtaposed with the genital haemal sinus along their basal surface (Davis, 1971; Smiley and Cloney, 1985). Moreover, echinoid oogenesis fails if the haemal connections of the ovary are severed (Okada, 1979; Pearse, 1982).

The dynamic changes in the genital haemal sinus of *Ophiolepis paucispina*, coincident with vitellogenesis, indicate that the haemal fluid may be a source of nutrients for oogenesis, as appears to be the case for several asteroid species (Walker, 1982). Furthermore, the prevalence of coated pits and vesicles along the oolemma indicates endocytotic uptake of exogenous material by the oocytes. The spike-like cytoplasmic coat of the pits is similar to that of pits involved in receptor-mediated endocytosis (Giorgi, 1980; Wild, 1980), and, based on the ultrastructural evidence of this study, it is suggested that yolk precursors may be selectively incorporated by the coated pits of *O. paucispina* oocytes. The haemal sinus appears to be the proximate source of these precursors which need only to pass through the basal lamina for access to the oocyte surface. Evidence for the presence of vitellogenic material in the genital haemal fluid of an asteroid species is reported in a recent immunocytochemical investigation (Beijnink *et al.*, 1984a). The presence of flocculent material around oocytes preserved with the cacodylate method suggests that haemal components may pass through the basal lamina. Furthermore, the material appears to be incorporated by the oocytes. It is not known if the absence of this material in oocytes fixed by the seawater method is due to extraction by the fixative.

As discussed in Byrne (1988), the nutritive material stored in the genital haemal sinus of *Ophiolepis paucispina* is presumably of somatic origin and is probably delivered to the ovary through the coelom. Coelomic translocation of nutrients and the absorptive properties of haemal fluid are demonstrated in several autoradiographic studies of asteroids where labelled amino acids were selectively concentrated by the haemal system (Ferguson, 1970, 1982, 1984; Beijnink and Voogt, 1984; Voogt *et al.*, 1985). Nutrient concentrations in echinoderm coelomic fluid is low (Ferguson, 1982; Beijnink *et al.*, 1984b) and it appears that the haemal sinus sequesters nutrients from coelomic circulation, and stores them, against a concentration gradient. Elevated concentrations of nutrients in the haemal sinus would also create a gradient favoring uptake of material by the oocytes. The apparent affinity of genital haemal fluid for nutrients and its differential accumulation around the vitellogenic oocytes of *O. paucispina* appear to be specialized features for a nutritive role in gametogenesis. The mechanisms underlying the dynamic properties of the haemal fluid, an extracellular material, remain to be elucidated.

Coated pits are short-lived (Dautry-Varset and Lodish, 1984), and the prevalence of endocytotic profiles in *Ophiolepis paucispina* oocytes suggests that incorporation of exogenous material is intense throughout vitellogenesis. Because the endosomes lose their extracellular coat soon after internalization and appear to fuse with pre-existing vesicles, the fate of the endocytosed material could not be followed. The material may be transferred directly to yolk bodies along with material synthesized by the oocytes or may be processed by the ER-Golgi complex. Endocytosis is reported for several echinoderm oocytes with coated pits typically located at the bases of microvilli (Takashima and Takashima, 1966; Bal, 1970; Holland, 1971; Ferrand, 1984; Smiley, 1988). By contrast, the absence of coated pits is noted for the oocytes of an ophiuroid (Bell, 1974) and several echinoid and asteroid species (Verhey and Moyer, 1967; Geary, 1978; Beijnink *et al.*, 1984c; Aisenshtadt and Vassetzky, 1986). However, the apparent absence of pits in these oocytes may be due to a low intensity of endocytotic uptake, which would limit the chances of finding coated pits. This is suggested for the asteroid *Asterias rubens* Linné, where the oocytes are known to incorporate exogenous material, but ultrastructural evidence of coated pits has not been found (Beijnink *et al.*, 1984c).

The foregoing speculations on the presence of vitellogenin in the genital haemal fluid and its pathway to the ovary of *Ophiolepis paucispina* are based on ultrastructural information alone and require verification by following the fate of labelled vitellogenin in this species with tracer techniques as in studies of insect and polychaete vitellogenesis (Giorgi, 1980; Fischer and Dhainaut, 1985; Baert and Slomianny, 1987). The prevalence of coated pits in the oocytes of *O. paucispina*, indicates that this ophiuroid may be a good subject for such an autoradiographic study.

In the terminology used to categorize vitellogenesis (Anderson, 1974; Eckelbarger, 1984; Wourms, 1987), yolk formation in *Ophiolepis paucispina* appears to be at least partially heterosynthetic with exogenous origin of the yolk precursors. However, the site of vitellogenin synthesis has not been determined. Free ribosomes and ribosomes associated with ER in the ooplasm may participate in the elaboration of yolk proteins (autosynthesis). Biochemical investigations of vitellogenin synthesis in echinoids demonstrate that yolk precursors are synthesized in the intestine, coelom, and gonad (Harrington and Ozaki, 1986; Ozaki *et al.*, 1986; Shyu *et al.*, 1986). Moreover, Harrington and Ozaki (1986) have shown that coelomocytes are a major source of vitellogenin in *Dendraster excentricus*. The accumulation of vitellogenin in the echinoid coelom provides further evidence for the coelomic transport of echinoderm yolk precursors (Harrington and Ozaki, 1986; Shyu *et al.*, 1986).

In summary, this investigation provides evidence for endocytotic incorporation of nutrients by the oocytes of *Ophiolepis paucispina* and adds to the accumulating evidence that the echinoderm genital haemal sinus is an intragonadal nutrient store (Walker, 1982; Ferguson, 1982, 1984; Kanatani and Nagahama, 1983; Voogt *et al.*, 1985). This, taken together with the biochemical evidence for the somatic and ovarian synthesis of vitellogenin (Harrington and Ozaki, 1986, Ozaki *et al.*, 1986; Shyu *et al.*, 1976) indicates that entirely autosynthetic yolk formation may be rare, whereas mixed auto- and heterosynthetic yolk formation may be common in the Echinodermata.

Acknowledgments

This work was supported by postdoctoral fellowships from the Harbor Branch Oceanographic Institution and the Smithsonian Institution. I thank Dr. K. J. Eckelbarger and Dr. G. Hendler for critically reading the manuscript and Dr. S. Smiley for constructive comments. Thanks also to Dr. V. B. Morris, Ms. J. Brodie, and Dr. D. McGrath who also read the manuscript and to Mr. Michael Kelly for technical assistance. This paper is contribution number 690 of the Harbor Branch Oceanographic Institution, number 236 of the Smithsonian Marine Station, Link Port, number 298 of the Galway School of Marine Science, and number 1 of the University College Galway EM Centre.

Literature Cited

Aisenshtadt, T. B., and S. G. Vassetzky. 1986. Fine structures of oocytes and accessory cells of the ovary in the starfish *Patiria (Asterina) pectinifera* at different stages of oogenesis after 1-methyladenine-induced maturation. *Dev. Growth Differ.* **28:** 449–460.

Anderson, E. 1974. Comparative aspects of the ultrastructure of the female gamete. *Intl. Rev. Cytol. Suppl.* **4:** 1–70.

Bal, A. K. 1970. Ultrastructural changes in the accessory-cells and the oocyte surface of the sea urchin *Strongylocentrous droebachiensis* during vitellogenesis. *Z. Zellforsch.* **111:** 1–14.

Bal, A. K., F. Jubinvill, G. H. Cousineau, and S. Inqué. 1968. Origin and fate of annulate lamellae in *Arbacia punctulata* eggs. *J. Ultrastr. Res.* **25:** 15–28.

Baert, J. L., and M. C. Slomianny. 1987. Heterosynthetic origin of the major yolk protein, vitellin, in a nereid, *Perinereis cultrifera* (polychaete annelid). *Comp. Biochem. Physiol.* **88B:** 1191–1199.

Beijnink, F. B., J. J. S. Broertjes, F. Brands, and P. A. Voogt. 1984a. Immunocytochemical demonstration of vitellogenic substances in the haemal system of the sea star, *Asterias rubens*. *Mar. Biol. Lett.* **5:** 303–313.

Beijnink, F. B., I. Van der Sluis, and P. A. Voogt. 1984b. Turnover rates of fatty acids and amino acids in the coelomic fluid of the sea star *Asterias rubens*: implications for the route of nutrient translocation during vitellogenesis. *Comp. Biochem. Physiol.* **78B:** 761–767.

Beijnink, F. B., and P. A. Voogt. 1984. Nutrient translocation in the sea star: whole-body and microautoradiography after ingestion of radiolabeled leucine and palmitic acid. *Biol. Bull.* **167:** 669–682.

Beijnink, F. B., and P. A. Voogt. 1986. The aboral haemal system of the sea star, *Asterias rubens* (Echinodermata, Asteroidea): an ultrastructural and histochemical study. *Zoomorphology* **106:** 49–60.

Beijnink, F. B., C. W. Walker, and P. A. Voogt. 1984c. An ultrastructural study of relationships between the ovarian haemal system, follicle cells, and primary oocytes in the sea star, *Asterias rubens*. *Cell Tiss. Res.* **238:** 339–347.

Bell, A. C. 1974. Histology and ultrastructure of *Acrocnida brachiata*. Ph.D. Thesis, Queen's University, Belfast.

Buckland-Nicks, J., C. W. Walker, and F. S. Chia. 1984. Ultrastructure of the male reproductive system and of spermatogenesis in the viviparous brittle-star, *Amphipholis squamata*. *J. Morphol.* **179:** 243–262.

Byrne, M. 1988. Evidence for endocytotic incorporation of nutrients from the haemal sinus by the oocytes of the brittlestar *Ophiolepis paucispina*. Pp. 557–563 in *Echinoderm Biology: Proceedings of the Sixth International Echinoderm Conference*, R. D. Burke, P. V. Mladenov, P. Lambert and R. L. Parsley, eds. Balkema, Rotterdam.

Chatlynne, L. G. 1972. An ultrastructure study of oogenesis in the sea urchin, *Stronglyocentrotus purpuratus*. Ph.D. Thesis, Oregon State University.

Chia, F. S. 1968. Some observations on the development and cyclic changes of the oocytes in a brooding starfish, *Leptasterias hexactis*. *J. Zool. Lond.* **154:** 453–461.

Cloney, R. A., and E. Florey. 1968. The ultrastructure of cephalopod chromatophore organs. *Z. Zellforsch.* **89:** 250–280.

Cobb, J. L. S. 1987. Neurobiology of the Echinodermata. Pp. 483–525 in *Nervous Systems in Invertebrates*, M. A. Ali, ed. Nata. ASI Series Plenium Press, New York.

Dautry-Varsat, A., and H. F. Lodish. 1984. How receptors bring proteins and particles into cells. *Sci. Am.* **250:** 52–58.

Davis, H. S. 1971. The gonad wall of the Echinodermata: a comparative study based on electron microscopy. M.Sc. Thesis, University of California, San Diego.

Eckelbarger, K. J. 1979. Ultrastructural evidence for both autosynthetic and heterosynthetic yolk formation in the oocytes of an annelid (*Phragmatopoma lapidosa:* Polychaeta). *Tissue Cell* **11:** 425–443.

Eckelbarger, K. J. 1984. Comparative aspects oogenesis in polychaetes. Pp. 123–148 in *Polychaete Reproduction: Progress in Comparative Reproductive Biology*, A. Fischer and H.-D. Pfannenstiel, eds. Gustav Fischer, Verlag.

Eddy, E. M. 1975. Germ plasm and the differentiation of the germ cell line. *Intl. Rev. Cytol.* **43:** 229–280.

Fell, H. B. 1946. The embryology of the viviparous ophiuroid, *Amphipholis squamata*, Delle Chiaje. *Trans. R. Soc. N. Z.* **75:** 419–464.

Ferguson, J. C. 1970. An autoradiographic study of the translocation and utilization of amino acids by starfish. *Biol. Bull.* **138:** 14–25.

Ferguson, J. C. 1982. Nutrient translocation. Pp. 373–393 in *Echinoderm Nutrition*, M. Jangoux and J. M. Lawrence, eds. Balkema, Rotterdam.

Ferguson, J. C. 1984. Translocative functions of the enigmatic organs of starfish-the axial organ, hemal vessels, Tiedmann's bodies, and rectal caeca: an autoradiographic study. *Biol. Bull.* **166:** 140–155.

Ferrand, J. G. 1983. Etude comparée de la vitellogenèse chez *Asterina gibbosa* Penn. et *Asterias rubens* L. (Echinodermes, Astérides) et signification des enzymes lysosomiques des oeufs. Ph.D. Thesis, University of Orléans.

Ferrand, J. G. 1984. Lysosomes and yolk formation in the oocytes of *Asterina gibbosa* (Echinodermata, Asteroidea). *Int. J. Inv. Reprod. Dev.* **7:** 143–147.

Fischer, A., and A. Dhainaut. 1985. The origin of yolk in the oocytes of *Nereis virens* (Annelida, Polychaeta) electron-microscopic and

autoradiographic studies by use of unspecific and yolk-specific markers. *Cell Tiss. Res.* **240:** 67–76.

Geary, E. D. 1978. Oogenesis in the Pacific sand dollar *Dendraster excentricus* (Eschscholtz). M.Sc. Thesis, University of Alberta.

Giorgi, F. 1980. Coated vesicles in the oocyte. Pp. 135–177 in *Coated Vesicles*, C. D. Ockleford and A. Whyte, eds. Cambridge University Press, Cambridge.

Harrington, F. E., and H. Ozaki. 1986. The major glycoprotein precursor in echinoids is secreted by coelomocytes into the coelomic plasma. *Cell Diff.* **19:** 51–58.

Hendler, G. 1975. Adaptational significance of ophiuroid development. *Am. Zool.* **15:** 691–715.

Hendler, G. 1979. Sex-reversal and viviparity in *Ophiolepis kieri*, n.sp., with notes on viviparous brittlestars from the Caribbean (Echinodermata: Ophiuroidea). *Proc. Biol. Soc. Wash.* **92:** 783–795.

Hendler, G., and P. A. Tyler. 1986. The reproductive cycle of *Ophioderma brevispinum* (Echinodermata: Ophiuroidea). *Mar. Ecol.* **7:** 115–122.

Holland, N. D. 1971. The fine structure of the ovary of the feather star *Nemaster rubiginosa* (Echinodermata: Crinoidea). *Tissue Cell* **3:** 161–175.

Holland, N. D. 1979. Electron microscopic study of the cortical reaction of an ophiuroid echinoderm. *Tissue Cell* **11:** 445–455.

Holland, N. D., J. C. Grimmer, and H. Kubota. 1975. Gonadal development during the annual reproductive cycle of (Echinodermata: Crinoidea). *Biol. Bull.* **148:** 219–242.

Holland, N. D., and H. Kubota. 1975. Fluctuations in the volume of non-germinal cell populations during the annual reproductive cycle of *Comanthus japonica* (Echinodermata, Crinoidea). *Annotn. Zool. Jpn.* **48:** 83–89.

Kanatani, H., and Y. Nagahama. 1983. Echinodermata. Pp. 611–654 in *Reproductive Biology of Invertebrates. Vol. 1: Oogenesis, Oviposition and Oosorption*, K. G. Adiyodi and R. G. Adiyodi, eds. Wiley and Sons, New York.

Kessel, R. G. 1966. Some observations on the ultrastructure of the oocyte of *Thyone briareus* with special reference to the relationship of the Golgi complex and endoplasmic reticulum in the formation of yolk. *J. Ultrastr. Res.* **16:** 305–319.

Kessel, R. G. 1968. An electron microscope study of differentiation and growth in oocytes of *Ophioderma panamensis*. *J. Ultrastr. Res.* **22:** 63–89.

Mladenov, P. V. 1986. Reproductive biology of the feather star *Florometra serratissima:* gonadal structure, breeding pattern, and periodicity of ovulation. *Can. J. Zool.* **64:** 1642–1651.

Norrevang, A. 1968. Electron microscopic morphology of oogenesis. *Intl. Rev. Cytol.* **23:** 113–186.

Okada, M. 1979. The central role of the genital duct in the development and regeneration of the genital organs in the sea urchin. *Dev. Growth Differ.* **21:** 567–576.

Ozaki, H., O. Moriya, and F. E. Harrington. 1986. A glycoprotein in the accessory cell of the echinoid ovary and its role in vitellogenesis. *Roux's Arch. Dev. Biol.* **195:** 74–79.

Patent, D. 1968. The general and reproductive biology of the basket star *Gorgonocephalus caryi*. PhD. Thesis. University of California, Berkeley.

Patent, D. H. 1976. Gonadal histology of the basket star, *Gorgonocephalus eucnemis*. *Thalassia Jugosl.* **12:** 269–276.

Pearse, J. S. 1982. Gametogenesis, aboral ring, axial complex, tube feet of an echinoid. P. 479 in *Echinoderms: Proceedings of the International Conference, Tampa Bay*, J. M. Lawrence, ed. Balkema, Rotterdam.

Piatigorsky, J. 1975. Gametogenesis. Pp. 42–98 in *The Sea Urchin Embryo*, G. Czihak, ed. Springer-Verlag, Berlin.

Rieger, R. M., and J. Lombardi. 1987. Ultrastructure of coelomic lining in echinoderm podia: significance for concepts in the evolution of muscle and peritoneal cells. *Zoomorphology* **107:** 191–208.

Schroeder, P. C., J. H. Larsen, and A. E. Waldo. 1979. Oocyte-follicle cell relationships in a starfish. *Cell Tiss. Res.* **203:** 249–256.

Shyu, A-B., R. A. Raff, and T. Blumenthal. 1986. Expression of the vitellogenin gene in female and male sea urchin. *Proc. Natl. Acad. Sci. USA* **83:** 3865–3869.

Smiley, S., and T. A. Cloney, R. A. 1985. Ovulation and the fine structure of the *Stichopus californicus* (Echinodermata: Holothuroidea) fecund ovarian tubules. *Biol. Bull.* **169:** 342–364.

Smiley, S. 1988. The dynamics of oogenesis in *Stichopus californicus* (Echinodermata: Holothuroidea), and an explanation of its annual ovarian cycle. *Biol. Bull.* **175:** 79–93.

Strathmann, R. R., and M. F. Strathmann. 1982. The relationship between adult size and brooding in marine invertebrates. *Am. Zool.* **119:** 91–101.

Takashima, Y. 1968. The ultrastructure of cortical granules with special reference to effects of fixation. *Med. J. Osaka Univ.* **19:** 95–111.

Takshima, Y., and R. Takashima. 1966. Electron microscope investigations on the modes of yolk and pigment formation in sea urchin oocytes. *Okajimas Folia Anat. Jpn.* **42:** 249–264.

Tyler, P. A. 1976. The ecology and reproductive biology of the genus *Ophiura* with special reference to the Bristol Channel. Ph.D. Thesis, University of Wales.

Verhey, C. A., and F. H. Moyer. 1967. Fine structural changes during sea urchin oogenesis. *J. Exp. Zool.* **164:** 195–226.

Voogt, P. A., J. J. S. Broertjes, and R. C. H. M. Oudejans. 1985. Vitellogenesis in sea star: physiological and metabolic implications. *Comp. Biochem. Physiol.* **80A:** 141–147.

Walker, C. W. 1974. Studies on the reproductive systems of sea-stars. 1. The morphology and histology of the gonad of *Asterias vulgaris*. *Biol. Bull.* **147:** 661–677.

Walker, C. W. 1979. Ultrastructure of the somatic portion of the gonads in asteroids, with emphasis on flagellated-collar cells and nutrient transport. *J. Morphol.* **162:** 127–162.

Walker, C. W. 1982. Nutrition of gametes. Pp. 449–468 in *Echinoderm nutrition*. M. Jangoux, and J. M. Lawrence, eds. Balkema, Rotterdam.

Wild, A. E. 1980. Coated vesicles: a morphologically distinct subclass of endocytotic vesicles. Pp. 1–24 in *Coated Vesicles*, C. D. Ockelford, and A. Whyte, eds. Cambridge University Press, Cambridge.

Wourms, J. P. 1987. Oogenesis. Pp. 50–178 in *Reproduction in Marine Invertebrates. Vol. 9 Seeking Unity in Diversity*, A. C. Giese, J. S. Pearse, and V. B. Pearse, eds. Blackwell Scientific, Palo Alto.

Yamashita, M. 1984. Electron microscopic observations on the cortical reaction of the brittle-star, *Amphipholis kockii* (Lutken), with special reference to its vitelline coat modification as revealed by the surface replica method. *Dev. Growth Differ.* **26:** 177–189.